PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

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Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
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International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/SE 03/00937	05.06.2003	05.06.2002
International Patent Classification (IPC) or both national classification and IPC		
G01N30/00		
Applicant		
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1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
18.12.2003	09.09.2004
Name and mailing address of the international preliminary examining authority:	Authorized Officer
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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/SE 03/00937

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-18 as published

Claims, Numbers

1-50 received on 24.08.2004 with letter of 24.08.2004

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-50
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-50
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-50
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: CHAURAND P; STOECKLI M; CAPRIOLI R M, ANALYTICAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY. COLUMBUS, US VOL 71(1999)5263-5270, "DIRECT PROFILING OF PROTEINS IN BIOLOGICAL TISSUE SECTIONS BY MALDI MASS SPECTROMETRY"

D2: STOECKLI M; CHAURAND P; HALLAHAN D E; CAPRIOLI R M, NATURE MEDICINE, VOLUME 7, NUMBER 4, APRIL 2001, 493-496 "IMAGING MASS SPECTROMETRY: A NEW TECHNOLOGY FOR THE ANALYSIS OF PROTEIN EXPRESSION IN MAMMALIAN TISSUES"

Documents D1 and D2 were not cited in the international search report.

Technical field:

The application is related to a method for identification and localization of biomolecules in a biological preparation.

Problem:
The problem to be solved is to analyse the spatial distribution of a chemical substance retained by a biological matter.

Solution:
A method according to claim 1, wherein a sample of biological matter is prepared by freezing, lyophilization, freeze-substituting or air-drying prior to a chemical imprint on a substrate to save the lateral distribution of the substances. Subsequent imaging mass spectrometry provides information about the spatial distribution of the chemical substances.

Prior art:

D1 discloses that samples are applied on a transfer membrane for protein blotting followed by mass spectrometry.
D2 discloses imaging mass spectrometry of frozen slices of tissue.

Novelty (Article 33(2) PCT):
The subject-matter of claim 1 differs from D1 in that samples are not in the wet state and in that mass spectrometry is performed with spatial resolution.
The subject-matter of claim 1 differs from D2 in that the sample is imprinted on a substrate.
Therefore the subject-matter of claim 1 is new over the available prior art.

Inventive step (Article 33(3) PCT):
Freezing, lyophilization, freeze-substituting or air-drying prior to a chemical imprint on a substrate is not hinted or suggested in the prior art. Therefore claim 1 meets the requirements of inventive step.

Claims 2-50 are dependent claims and therefore they meet the requirements of the PCT too.

1. A method of analyzing the spatial distribution of at least one chemical substance retained by a biological matter, comprising the steps of
   a) supplying a sample of said biological matter having a specimen surface;
   b) supplying said sample of biological matter having a specimen surface in a frozen state, or subjecting said sample to lyophilisation, freeze-substitution or air-drying;
   c) producing at least one imprint of said specimen surface on at least one corresponding separate substrate surface;
   d) subjecting said at least one imprint on said substrate surface to imaging mass spectrometry, at least one signal being produced from an array of points on said substrate surface,
   e) recording said at least one signal from an array of points as at least one image; and
   f) determining said spatial distribution of said at least one chemical substance retained by the biological matter from said at least one image of said at least one imprint.
2. The method according to claim 1, wherein said at least one chemical substance comprises organic material.
3. The method according to claim 2, wherein said organic material comprises a lipid, an amino acid, a peptide, a protein, a carbohydrate, a nucleotide, a transmitter substance, a drug, or a targeting molecule.
4. The method according to claim 3, wherein said targeting molecule is a complementary DNA-sequence.
5. The method according to claims 3-4, wherein said targeting molecule is an antibody or a fragment thereof.
6. The method according to claim 5, wherein said targeting molecule comprises a chemical label.
7. The method according to claim 6, wherein said chemical label is an unusual element or an isotope.
8. The method according to claim 3, wherein said nucleotide is a DNA-molecule.
9. The method according to any of the preceding claims, wherein said biological matter comprises cells, tissue, virus, body liquid, or biological molecules.

10. The method according to any of the preceding claims, wherein said sample of said biological matter is supplied as a specimen surface *in situ*.
11. The method according to any of preceding claims, wherein said sample of said biological matter is provided as a specimen surface by applying it on a solid surface.
12. The method according to claim 11, wherein the solid surface is a glass surface.
13. The method according to any of preceding claims, wherein multiple sequential imprints are produced from said specimen surface.
14. The method according to any of preceding claims, wherein said biological matter is fractured or cut in order to expose its interior before producing said at least one imprint.
15. The method according to any of preceding claims, wherein said specimen surface is pretreated immediately before producing said at least one imprint.
16. The method according to claim 15, wherein said specimen surface is pretreated by condensing a liquid of a non-polar solvent and/or a polar solvent onto the same.
17. The method according to claim 16, wherein said polar solvent is a water solution.
18. The method according to any of claims 16-17, wherein said specimen surface is first brought to room temperature or cooled and is then arranged above a heated container containing said liquid.
19. The method according to any of claims 15-18, wherein said at least one imprint is produced within 100 s after said pretreatment of said specimen surface.
20. The method according to any of preceding claims, wherein said specimen and/or said substrate is flexible.
21. The method according to any of preceding claims, wherein said substrate surface is a metal surface.
22. The method according to claim 21, wherein said metal surface is a silver, gold, palladium, platinum, nickel, chromium, or a copper surface.
23. The method according to claim 22, wherein the metal surface is silver.

24. The method according to any of preceding claims, wherein said substrate surface is structured.
25. The method according to claim 24, wherein said substrate surface is structured with protrusions of 0.01-5 μm.
26. The method according to any of preceding claims, wherein said substrate surface is polished.
27. The method according to any of preceding claims, wherein said substrate surface is cleaned immediately before producing said at least one imprint.
28. The method according to claim 27, wherein said substrate surface is cleaned by means of chemical etching, plasma cleaning, or UV/ozone treatment, or a combination thereof.
29. The method according to any of preceding claims, wherein said biological matter is subjected to a salt solution before and/or after providing said sample of biological matter as a specimen surface.
30. The method according to claim 29, wherein said salt is a sodium salt, a potassium salt, a copper salt or a silver salt, preferably a silver salt.
31. The method according to any of preceding claims, wherein said at least one imprint is produced by pressing said specimen surface against said substrate surface.
32. The method according to claim 31, wherein said pressing is accomplished by means of a compressible material.
33. The method according to claim 31-32, wherein said pressing is accomplished by applying a force between 0.01 and 10 MPa.
34. The method according to claim 33, wherein said pressing is performed for up to 100 s.
35. The method according to any of claims 31-34, wherein said pressing is performed so that said at least one imprint represents below 5 monolayers, preferably below 2 monolayers.
36. The method according to any of the claims 21-28, wherein a metal layer is deposited onto said substrate surface before producing said at least one imprint.
37. The method according to any of the claims 1-36, wherein a metal layer is deposited onto said substrate surface after producing said at least one imprint.
38. The method according to claim 37, wherein said layer of metal has a thickness of less than 100 nm.

39. The method according to claims 36-38, wherein said layer of metal is a silver layer.
40. The method according to any of preceding claims, wherein said imaging mass spectrometry is a Secondary Ion Mass Spectrometry.
41. The method according to claim 40, wherein said Secondary Ion Mass Spectrometry is Time of Flight - Secondary Ion Mass Spectrometry.
42. The method according to any of claims 40- 41, wherein a focused beam of ions is produced by the primary ion source in said Secondary Ion Mass Spectrometry.
43. The method according to claim 42, wherein said ions are C_{60}, Ga, In, or Au ions.
44. The method according to claim 43, wherein said Au ions are clusters of n ions, $n \leq 10$.
45. The method according to any of claims 42-44, wherein said focused beam has a diameter below 10 μm, preferably below 1 μm.
46. The method according to any of preceding claims, wherein a light sensitive matrix is applied onto said substrate surface before or after producing said at least one imprint.
47. The method according to any of preceding claims, wherein a light sensitive matrix is applied onto said specimen surface before producing said at least one imprint, a portion of said light sensitive matrix being transferred to the substrate surface when said at least one imprint is produced.
48. The method according to any of preceding claims, wherein said imaging mass spectrometry is a Matrix Assisted Laser Desorption Ionization.
49. The method according to any of preceding claims, wherein the light source of said Matrix Assisted Laser Desorption Ionization comprises a focused laser beam, preferably an ultraviolet laser beam.
50. The method according to any of preceding claims, wherein said at least one image is produced from said at least one signal, the color or the brightness in each point of said at least one image being dependent on the magnitude of said at least one signal from the corresponding point on said substrate surface.